Short Communication

Recovery of melanized yeasts from Eastern Mediterranean beach sand associated with the prevailing geochemical and marine flora patterns

MARI A. EFSTRATIOU* & ARISTEA VELEGRAKI†

*Department of Marine Sciences, University of the Aegean, University Hill, Mytilene, and †Mycology Laboratory, Microbiology Department, Medical School, National and Kapodistrian University of Athens, Greece

The melanized opportunistic pathogens *Exophiala dermatitidis* (Chaetothyriales) and *Aureobasidium pullulans* (Dothideales) were sporadically isolated from beach sand. This is the first time they are reported from bathing beach sand, thus providing updates on the conditions influencing the in situ black yeast community structure and raising public health concerns.

**Keywords** *Aureobasidium pullulans, Exophiala dermatitidis, beach sand*

---

**Introduction**

The last two decades are marked by the changing epidemiology of serious hospital and community-acquired opportunistic cutaneous and invasive mycoses of immunocompromised hosts [1,2]. The changing epidemiological trends do not only refer to the variety of species causing common opportunistic infections such as candidiasis, aspergillosis or cryptococcosis [2], but also to new and emerging fungal pathogens. Among these, black yeasts have taken their toll in human life and welfare [3–5].

The phylogenetically diverse melanized meristematic fungi are geographically distributed world-wide and belong, or are closely related, to at least three different orders of the fungal Kingdom, Chaetothyriales, Dothideales and Pleosporales. They occur epilithically or endolithically [6], in environments with low water availability and can withstand high osmotic stress as well as extreme temperatures. Their thick cell wall confers protection from desiccation [6] and melanin protection from UV [7]. Isolation of the halotolerant *A. pullulans* is reported from marine ecosystems, salt marshes and hypersaline salterns [8], whereas *E. dermatitidis* has been isolated from marble and granite [6]. They have been described as etiological agents of infections in healthy and immunocompetent individuals following traumatic implantation [7,9] and are long known to be associated with a broad spectrum of diseases that range from localized infections to allergies and brain abscess in immunocompromised individuals [5,7,9–11].

Thus far, there is limited information on the occurrence of any kind of yeasts in bathing beach sand [12–14]. As infections can be caused through accidental traumatic implantation, investigation of black yeast occurrence in environments used for recreational activities is of public health significance. In that respect, we have screened sand specimens for presence of black yeasts from four marine bathing beaches on the island of Lesvos, Greece.

**Materials and methods**

Sixty samples were collected from four beaches located on the eastern coast of the island of Lesvos, northeast Aegean Sea, in the Mediterranean. Tsamakia beach is situated approximately 1 km north of the port of Mytilene, a city of 30,000 inhabitants. Thermi/Kanoni beach and Pamphila/Niselia beach are located 16 km and 12 km to the north, and Varia beach is 5 km to the south of Mytilene. The beaches were sampled once a fortnight from March to October. Samples of wet sand were aseptically removed from the surface to approximately 5 cm depth and transported to the laboratory within one hour of collection. Two grams of each sample were transferred into 9 ml selective.
broth medium supplemented with 0.05% chloramphenicol (Sigma, St Louis, MO, USA), prepared as proposed by de Hoog and Haase [15]. The tubes were briefly vortexed and incubated at room temperature for 72 h. Then 1 ml suspension from each tube was spread on Malt Extract Agar (Difco, Lawrence, Kansas, USA) and incubated at room temperature for 7–15 days. Dark brown/black colony forming units (cfu) were identified by morphology, physiology and sequence (Macrogen, Seoul, Korea) of the rRNA internal transcribed spacer (ITS) regions as described before [16]. The isolates under *A. pullulans* HCPF 5673b and *E. dermatitidis* HCPF 5674b are deposited in the UOA/HCPF1 collection (http://wdcm.nig.ac.jp/hpcc.html).

### Results and discussion

Black yeasts were isolated in 3/60 sand specimens in 3/4 sites (Table 1). Conventional, morphology and physiology studies and molecular identification of our *A. pullulans* (GenBank Acc. No. FJ232892) and *E. dermatitidis* (GenBank Acc. No. FJ387565) were congruent. Sequence analysis of the amplified ITS regions of both yeasts displayed 99–100% homology with the published (http://www.ncbi.nlm.nih.gov) ITS 1, 5.8S and ITS 2 sequences of *A. pullulans* and *E. dermatitidis* respectively.

In the last two decades modern medical practices increased the numbers of immunocompromised individuals and brought about a corresponding increase in the cases of opportunistic mycoses followed by an increase in emerging fungal pathogens. Among them, *A. pullulans* is a ubiquitous oligotrophic, halotolerant saprophyte [7,8]. It is the cause of a variety of injury-associated localized infections in healthy, immunocompetent individuals and invasive infections in immunocompromised patients [7]. *E. dermatitidis* occurs in soil, fresh water and deep-sea hydrothermal fields [7,17]. It is the causative agent of nosocomial infections in diabetic, HIV-positive and hematology/oncology patients. Subclinical pulmonary non-invasive infections are reported in hospitalized and non-hospitalized cystic fibrosis patients [5], whereas bronchiectasis is observed in non-cystic fibrosis individuals [3].

Although *A. pullulans* has been reported in Brazil from sand dune ecosystems [18], and *E. dermatitidis* from Mid-Atlantic Ridge hydrothermal fields, to our knowledge this is the first time that it is reported from sand of recreational beaches. Use of selective enrichment media [15], an initial 72 h enrichment period in selective broth and the longer incubation periods required for growth, may have promoted their previously undetected recovery from beach sand. In our study, black yeasts were not detected in every sand specimen from the three sites (Table 1). Their sporadic isolation is attributed to the micro-tidal environment of the east coast of the island and to offshore extreme wave height of 1.55 m at 4.9 s period due to coastal waves generated by conventional ships and high-speed passenger ferries [19], which can cause rapid changes in the yeast microflora of the wet sand. In Mytilene’s Tsamakia beach this wave action might have also transported to the beach sediments with adsorbed hydrocarbons from the nearby port [20]. As hydrocarbons are important nutrients for *Exophiala* [21], this could be an additional factor favouring its occurrence. Isolation of *A. pullulans* and *E. dermatitidis* on two sampling occasions is also likely to have been facilitated by dead sea-grass (*Posidonia*) accumulations washed ashore from the seabed meadows. Forming a barrier to high coastal waves *Posidonia* accumulations, coinciding with the April and August sampling occasions (Table 1), may have prevented washing away of the black yeast microflora.

The occasional recovery of *Aureobasidium* and *Exophiala* species denotes that they constitute a transient black yeast flora of the wet sand at the three sampling sites. The prevalent geological, chemical and distinctive chemical attributes – such as the increased radon concentrations [22] – as well as coastal and marine flora factors (Table 1), predispose these sites for oligotrophic black yeast

### Table 1  Recovery of *Aureobasidium pullulans* and *Exophiala dermatitidis* from sand specimens in relation to the geological, chemical, coastal and sea flora patterns at the isolation sites

<table>
<thead>
<tr>
<th>Recovery date/Black yeast</th>
<th>Location/Beach</th>
<th>Beach characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 April 2004 <em>E. dermatitidis</em></td>
<td>Mytilene/Tsamakia</td>
<td>Pleistocene limestone and basaltis. Surficial coastal sediments contain polycyclic aromatic hydrocarbons (PAH 346.0 ng/g dw) [20], due to deposits from ship docking at the nearby main port of the island.</td>
</tr>
<tr>
<td>25 July 2004 <em>A. pullulans; E. dermatitidis</em></td>
<td>Thermi/Kanoni</td>
<td>*Igneous marble, limestone sand. Site in close proximity to thermal springs resulting from mixing meteoric and infiltrating seawater. Thermal spring water with redox sensitive metals and increased radon concentrations (20–60 kBq.m⁻³) [22].</td>
</tr>
<tr>
<td>03 August 2004 <em>A. pullulans</em></td>
<td>Pamphila/Nisselia</td>
<td>*Igneous marble and basaltis, limestone sand; small river – dry during the summer – drains on beach agricultural and pastoral land wastes in winter and spring.</td>
</tr>
</tbody>
</table>

*Dead sea-grass (*Posidonia*) accumulations at sampling sites.*
colonization. It is acknowledged that environments high in salinity, rich in vegetation, limestone and hydrocarbons provide a suitable substrate for meristematic melanized yeasts [6–8]. In that respect even the temporary presence of potential pathogens in wet sand of recreational beaches raises public health concerns, thus instigating surveillance programs and subsequent implementation of control measures.

Acknowledgements

Supported by the University of the Aegean Research Unit fund 1972 and the National and Kapodistrian University of Athens SARG 70/4/5905 and 70/3/6915.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Note

1. World Data Centre of Microorganisms: WDCM929.

References


